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This study quantified the frequency of simple versus complex races of *Puccinia striiformis* Westend. in mixtures of wheat cultivars possessing different race-specific resistance genes. A simple race of a pathogen can infect only one component, and a complex race of the pathogen can infect two or more components of an intraspecific plant mixture. The treatments were designed so that the race that was complex changed depending on the host mixture, thus enabling us to observe the influence of pathogen complexity in different host genetic backgrounds. Six cultivar mixtures and one pure stand of winter wheat were inoculated with three races of *P. striiformis* (CDL 27, CDL 29, and CDL 41) at two locations for two seasons. Potted plants of three winter wheat cultivars (Paha, Tres, and Tyee) that were each susceptible to one of the three races of the pathogen were used to sample the pathogen during the field epidemics. Disease incidence on the differential cultivars

was used to calculate the proportion of the three races in each treatment. The specific cultivars included in the mixtures influenced the frequencies of the three races. Increasing the number of virulent races in a mixture reduced the frequency of the complex race relative to the other two races. When two of the races (races 29 and 41) were complex on the same mixture, location had an effect on which of the races was more frequent. When race 29 was the complex race in the mixture, it was more frequent than when race 41 was the complex race. The results suggest that environmental interactions, genetic background of the pathogen race, host composition, and interaction among pathogen races may be as important in determining race frequencies in mixtures as is stabilizing selection *sensu* Vanderplank (1968).

Effect of Wheat Cultivar Mixtures on Populations of *Puccinia striiformis* Races

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Chapter 1

INTRODUCTION

The use of crop monocultures has led to what Browning and Frey (1969) describe as a vicious circle. Commercial cultivars are bred to be resistant to the most prevalent pathogen races. After their release, virulent pathogen genotypes are selected from within the existing population, or arise as new mutants, and render the resistance ineffective. Subsequently, new cultivars are developed, with the same result. Although some cultivars seem to have durable resistance *sensu* Johnson (1979), others may lose their resistance in as little at two years.

In natural ecosystems, as well as in traditional agricultural systems, the host population is more diverse (Mundt & Browning, 1985; Wolfe, 1985). Mundt and Browning (1985) refer to the diversity of natural ecosystems as a functional diversity (*sensu* Schmidt, 1978), in that coevolution has selected for the diverse host and pathogen populations that reduce disease severity. In the center of coevolution for the rusts and small grains, epidemics are rare (Browning, 1974). Traditional agricultural systems use intercropping as well as cultivar mixtures to diversify the crop population in order to buffer the crop from stresses

and to increase the chances of obtaining an acceptable yield (Wolfe, 1985).

Jensen (1952) described the need for diversification to "re-establish genetic barriers in the form of resistant varieties" in order to impede the spread of plant pathogens. He suggested the development of a multiline cultivar that would be a combination of phenotypically uniform cultivars that differ in a few genetic factors, such as disease resistance. "A multiline variety would be expected on theoretical grounds to possess the characteristics of longer varietal life, greater stability of production, broader adaptation to environment, and greater protection against disease" (Jensen, 1952). Borlaug (1959) developed a multilineal cultivar to aid in the control of stem rust in Mexico. Commonly grown commercial cultivars were used as recurrent parents in crosses with cultivars that contained resistance genes for stem rust. In most cases, two or three backcrosses were all that were necessary to produce lines that were uniform for agronomic traits. The individual lines were then tested for their reactions to stem rust races. The best of these lines were to be mixed to form the multiline, the components of which could be replaced if their resistance was overcome (Borlaug, 1959). However, during the development of this multiline new agronomically superior cultivars were developed, making the cultivars used in the multiline agronomically less advantageous (Browning &

Frey, 1969).

Wolfe (1985) described the advantages of using cultivar mixtures instead of multilines. Heterogeneous mixtures should provide reliable yield due to compensation in the performance of some of the components for the poor performance of others. "Cultivar mixtures provide a greater potential for practical application" because of the larger number of cultivars that can be used in the mixtures, because cultivar mixtures are easily adaptable to differences and changes in agronomic practices, and because components of a mixture can be easily replaced if their resistance becomes ineffective. Wolfe also felt that, the more genetically diverse the host population, the more difficult it would be for the pathogen population to increase its pathogenicity.

Many field trials have shown that cultivar mixtures reduce disease severity in a given year (Chin & Wolfe, 1984a; Wolfe, 1985; Mahmood et.al., 1991; Finckh & Mundt, 1992a). However, the potential for the widespread use of cultivar mixtures has lead to concern over the development of pathogen races capable of infecting most or all of the components of the mixture. Races that can infect two or more components of a mixture are known as complex races. If such complex or super races were able to develop faster on a heterogeneous host than on a pure line cultivar, the use of mixtures may not lead to improved disease control and

longer varietal life. Vanderplank (1968) first discussed the problem of complex races with regard to the deployment of different resistance genes over a geographic area. After reviewing data from studies of different pathosystems, he concluded that races with unnecessary genes for virulence on a specific cultivar (i.e., virulence genes not needed to induce disease on that cultivar) will be less fit on that cultivar than races without unnecessary virulence genes. The deployment of a resistance gene will lead to selection for virulence to this resistance gene, which Vanderplank referred to as directional selection. Once the resistance is overcome the use of this cultivar will most likely decrease, resulting in stabilizing selection against the now unnecessary virulence in the pathogen population (Vanderplank, 1968).

Many mathematical models have been proposed to predict the frequency of complex races of the pathogen in mixed host populations. Most of these have assumed a cost of fitness to a race of the pathogen with unnecessary virulence. The results of Leonard's models and field work (1969a & 1969b) suggest that a mixed host population can act to stabilize the pathogen population if the host population contains a percentage of cultivars susceptible to all races of the pathogen. Leonard calculated this percentage to be 35-40%. He also found evidence for interaction between two races of the pathogen when they were both virulent on the same

component of the mixture. The amount of rust on the cultivar in the mixture that was susceptible to two races of the pathogen was less than the sum of the amount of rust on susceptible cultivars grown separately. However, when the two races infected different components of the mixture the two races did not interfere with each other (Leonard, 1969a).

Barrett (1978) proposed a model for epidemic development in cultivar mixtures that considered the frequency of individual races as well as the size of the whole pathogen population. The model contained two hosts each with a single different gene for resistance, and four races of the pathogen. One of the races had no virulence on the mixture, one could infect both components of the mixture, and two could each infect one component. In one of the four simulations, Barrett reduced the values assigned to the parameter *s* which represents selection against unnecessary virulence. This was the only situation in which the complex race was selected for, although the population size of the pathogen was still reduced.

Other mathematical models have predicted that mixtures may be protected from complex races due to stabilizing selection, as well as other factors. Leonard and Czochor (1980) concluded from a review of many models that the type of selection imposed on pathogen races will play a role in determining the effectiveness of multilines.

> "If selection in pathogen populations is, at least, partly soft selection that depends on competitive interactions (among races)... it might be possible to use multiline varieties to stabilize pathogen populations with a diversity of races in stable equilibria. However, if selection coefficients in pathogen populations depend on the intensity of competitive interactions, it is likely that selection against unnecessary genes for virulence will be more intense when the pathogen population densities are high than when they are low." (Leonard & Czochor, 1980)

Barrett (1980) concluded from his model simulations that several factors may be involved in the composition of the pathogen population on a mixed host. These include the reproduction rate of the pathogen, the proportion of each host component, and the fitness of the pathogen genotypes on each host component. He also concluded that 'super races' will not necessarily dominate the population, although they may increase initially because their diversity of virulence may give them an advantage during initial infection. Marshall (1989) also concluded that complex races may be at an advantage at the beginning of the season because a complex race can infect more plants in the mixture, but after initial infection, simple races should have a selective advantage. According to Marshall, stabilizing selection must be very high to select against complex races, and "will vary between loci, between environments, and between backgrounds."

Parlevliet (1981) gave a thorough review of studies concerning stabilizing selection. In many cases, genes for

virulence existed in populations where the corresponding resistance genes had never been introduced or were no longer used commercially. Parlevliet believed that although these genes were no longer the major source of resistance, they may still have been present in newer cultivars. In most of the studies, unnecessary genes for virulence remained in the population, and complex races were more frequent than simple races. "It was considerably more difficult to find recent examples in which virulence did not occur in excess."

Parlevliet (1981) concluded that stabilizing selection is a weak concept, and that the fitness of a pathogen is a factor of its environment. As an explanation for the results of Vanderplank (1968) and others who found simple races to be more fit than complex races, Parlevliet hypothesized that the pathogen adapts to new resistance genes in two stages. During the first stage, the pathogen becomes capable of infecting the new cultivar, but appears to be less competitive, the manifestation of which is a decrease in reproduction. It is during this first stage that Vanderplank made his observations. However, Parlevliet believes that, given enough time, the pathogen will regain its fitness.

Few field studies of pathogen race dynamics in host mixtures have been done. Chin and Wolfe (1984a) studied the response of *Erysiphe graminis* to pure and mixed stands of barley. They observed a reduction in disease severity in

the mixtures relative to the mean of the pure stands. Overall, complex races did not dominate the populations, nor were they eliminated. The complex races were observed to have a lower reproduction rate on pure stands of barley, but on some mixed host populations the complex races did dominate due to their virulence advantage. Chin and Wolfe concluded that selection for complex races in mixed host populations will be slow because complex genotypes are less able to adapt to individual hosts. The absolute frequencies of these genotypes will be reduced due to a reduction in the overall size of the pathogen population on a mixed host.

Crandall (1987) studied populations of *Rhynchosporium secalis* on composite cross populations of barley. Since the genetic composition of a composite cross population is unknown, she defined a complex race by the number of differentials it could infect, rather than by the number of components of the mixture it could infect. Complex races did not occur in high frequencies and were less common than simple races on most of the host populations, although the number of complex races did increase over the two-year period. The number of complex races varied between years and sites. Disease pressure also varied from site to site, and the site with the highest disease pressure also had the highest frequency of complex races (Crandall, 1987).

Huang, Kranz, and Welz (1990) studied powdery mildew (*Erysiphe graminis* f. sp. *hordei*) using three cultivars of

barley as differentials. All three had two resistance genes; resistance gene Mlg and either Mla12, Mlav, or Mla9. "There is a negative association between (the corresponding virulence genes) va 12 and vv and va12 and va9," meaning that va12 occurs with either vv or va9 at low frequencies. Differentials were inoculated in the lab using inoculum collected from the field. Complex races were more commonly found in the random barley mixtures, and their population increased during the epidemic. Even though Huang et. al. believed that the use of cultivar mixtures may lead to an increase in the frequency of complex races, they felt that the benefits of mixtures for disease control will outweigh this. If a multiline is capable of lowering the amount of inoculum produced by the pathogen population, be it simple or complex, the overall disease incidence will be lowered. Just as purelines of resistant cultivars are replaced, components in a mixture can be changed to slow down selection for complex genotypes in the pathogen population (Huang et.al., 1991).

The use of cultivar mixtures of winter wheat (*Triticum aestivum* L.) is gaining acceptance in the Pacific Northwest of the United States. One of the most prevalent diseases in the region is stripe rust, which is caused by the fungus *Puccinia striiformis* Westend. Cultivar mixtures with a diversity of resistance genes may prolong the usefulness of those resistance genes by slowing the rate at which the

pathogen can adapt. However, if complex races have some selective advantage on mixed host populations, the widespread use of cultivar mixtures could lead to an increase in the rate at which resistance genes are overcome. The frequencies of three races of *P. striiformis* were observed in six club wheat (*Triticum aestivum*) mixtures. Whether each of the three races was complex or simple was determined by the cultivars included in the different mixtures. The purpose of this study was to quantify the frequency of simple and complex races of *Puccinia striiformis* in mixtures of wheat cultivars possessing different race-specific resistance genes.

CHAPTER II

MATERIALS AND METHODS

Greenhouse experiments

A greenhouse experiment was conducted to determine the range of disease incidence at which the frequency of *P. striiformis* races could be accurately determined on differential cultivars. Twenty-five seeds each of the wheat cultivars Paha, Tres, Tyee, and Orin were planted in 10-cm plastic pots using a peat/perlite potting mix. The plants were fertilized with a 15-30-15 liquid fertilizer once per week beginning two weeks after planting, and were inoculated after three weeks of growth. Orin is susceptible to all three races and was used to determine if the inoculation was effective. For the purposes of this study, a cultivar was considered susceptible to a race of *P. striiformis* only if that race of the fungus could produce sporulating lesions on that cultivar. Differential susceptibilities of the other three cultivars allowed identification of the three races (CDL 27, 29, and 41) to be used for inoculation in the field (Table 1). The CDL race designations are those of the USDA Cereal Disease Laboratory, Pullman, WA, USA. A mixture of spores of the three races in equal amounts was used to inoculate at five spore concentrations; 0.0004, 0.001, 0.004, 0.02, and 0.04 total g of spores/pot. The spores were increased on susceptible plants in growth chambers.

Three pots were inoculated at each concentration with a mixture of spores and talc. The plants were placed in a dew chamber for 12 hours. Disease incidence readings were taken when lesions sporulated, which occurred after three weeks' incubation in the greenhouse. The second and third leaves of each plant were inspected for sporulating lesions. The number of infected second or third leaves divided by the total number of second or third leaves, respectively, gave disease incidence for each of the three races. The average percentage of diseased leaves was calculated for each concentration. The data for disease incidence were corrected for multiple infections using Gregory's multiple infection transformation (Gregory, 1948; Waggoner & Rich, 1981). The data for each race were then converted to a proportion of the total amount of infection for the three races. Fisher's protected L.S.D. means comparison procedures were used to compare the disease incidence values for the three races.

The second greenhouse experiment tested whether the sampling method used in the field would detect different frequencies of the three races. Each pot contained 25 plants each of Paha, Tres, and Tyee, and Hyak. The plants were maintained in the manner described above, except that a single application of a 17-6-10 slow-release fertilizer was used. Plants were inoculated at three weeks after planting with three ratios of races 29, 41, and 27: 2:1:1, 1:2:1, and

1:1:2. Based on results from the first greenhouse experiment, the inoculation rate was 0.02 g spore/talc mix per pot. Each ratio of spores was applied to six pots, and the experiment was repeated to give a total of twelve pots per ratio. The inoculation, disease evaluation, and statistical procedures were the same as those described above for the first greenhouse experiment. However, only the third leaves were used for disease assessment because the second leaves had senesced.

Field experiments

Experimental design and planting. This experiment was conducted at the Columbia Basin Agricultural Research Center field stations in Moro and Pendleton, Oregon, during the 1991 and 1992 winter wheat seasons. The two stations are 155 km apart and are located in semiarid, eastern Oregon. In 1991, 415 mm and 222 mm of precipitation fell at Pendleton and Moro, respectively. In 1992, Pendleton received 359 mm and Moro received 225 mm of precipitation. Planting dates were 19 September and 2 October 1990 (for the 1991 growing season), and 24 September and 7 October 1991 (for the 1992 growing season), at Moro and Pendleton, respectively. The seeding rate was 85 viable seeds/m^2 in Pendleton, and 66 viable seeds/m^2 at the drier site in Moro. Fertilization, as well as insect and weed control, was typical for conventional wheat production at each location.

In 1991, data were obtained from a larger experiment

consisting of pure stands of six club wheat cultivars (Hyak, Jacmar, OR855, Paha, Tres, and Tyee) and ten mixtures of those cultivars. The experiment was in a split plot design with four replications. The main plots were two disease treatments, either inoculated with stripe rust or not inoculated and treated with fungicide. Subplots consisted of the sixteen wheat populations. Each subplot was 1.5 x 4.3 m. A subplot of the stripe rust-resistant, common soft white winter wheat Stephens was planted between each subplot in the long dimension, and there was 1.8 m of fallow ground between subplots in the narrow dimension. There was a 6.1-m border of Stephens wheat planted between the main plots and replications. Stripe rust races were sampled from eight wheat populations (Table 2) in the inoculated main plots. Since only experimental units from inoculated main plots were utilized in the present study, we will refer to inoculated subplots simply as "plots" for the remainder of the paper.

In 1992, there were no fungicide-treated plots. The treatments were those shown in Table 2 plus one additional pure stand and two mixtures not related to the present work. The experiment was planted in a randomized complete block design with four replications. Each plot was 1.5 x 4.9 m. There were two plots of Stephens wheat planted as buffers between plots in the narrow dimension, and 1.2 m of fallow ground between plots in the long dimension. Treatment and

buffer plots were staggered in a checkerboard design. There was an additional 6.1-m of Stephens planted between each of the four replications.

Inoculation. All plots were inoculated with races CDL 27, 29, and 41 of *Puccinia striiformis*, which show differential virulence to the wheat cultivars used in the experiments (Table 1). Races 29 and 41 were either complex (able to infect two cultivars in the mixture) or simple (able to attack only one cultivar in the mixture) depending on the cultivars included in each mixture (Table 2). The mixtures were designed to clarify whether the frequency of a race was due to complexity for virulence, or to some other aspect of its genetic background. These treatments allowed a comparison of the reproduction of two races when both are complex, when one is complex, or when both are simple. Finally, the mixtures allowed a comparison of the reproduction of complex races in the presence of either one or two simple races.

All three races of the pathogen were maintained on susceptible cultivars in growth chambers. Peat pots containing two-to-four plants of the cultivar Nugaines, which is susceptible to all three races in the seedling stage, were inoculated with an equal number of spores of all three races. The pots were then placed in a dew chamber overnight, and, subsequently, placed in a cold frame to acclimate them to outdoor temperatures. Upon sporulation,

the plants were transplanted into the field plots. Two peat pots were transplanted into the center of each plot on 18 and 19 March at Moro and Pendleton, respectively, in both 1991 and 1992. The spreader plants were removed from the plots one week before sampling began.

Race evaluation. Twenty-five plants each of four cultivars of wheat (Hyak, Paha, Tres, and Tyee) were planted in 10-cm plastic pots using a peat/perlite potting mix. The plants were fertilized weekly from two weeks after planting with a 15-30-15 liquid fertilizer in 1991, and a single application of a 17-6-10 slow-release fertilizer in 1992. The plants were maintained in the greenhouse for three weeks, after which time they were placed in the field plots to sample the pathogen. The three races used for inoculation could each infect one of the differential cultivars (Table 1). A cultivar was considered susceptible to a race of the fungus only if that race could produce sporulating lesions on that cultivar. Sampling took place every other week, beginning 5 wk after the initial inoculation of the plots. Four visits to each site were made each year. In 1991, all four visits yielded sufficient infection for data analysis at Pendleton, but at Moro there was insufficient infection from the second visit. Due to drought conditions during the spring of 1992, the wheat matured more quickly, and only three sampling visits yielded results at Pendleton, and only two at Moro.

In order to obtain an optimum level of infection, replicate sets of pots were left in the field for different amounts of time; e.g., 1, 2, 4, and 6 hours. The pots were placed in a dew chamber overnight, and then were placed in the greenhouse. After approximately three weeks, when the lesions were sporulating, disease incidence readings were taken. Pots placed in the pure stands of Jacmar (the most severely diseased treatment) were inspected to determine which of the four time periods allowed for adequate infection. If infection on the trap plants from the Jacmar plots was above 20%, all pots from that time period were read by inspecting the third leaf of each plant for the presence of sporulating lesions. The total number of third leaves with sporulating lesions divided by the total number of third leaves for that cultivar gave disease incidence for each of the three races. In some cases, data from one time period was not used because sporulating lesions were only found on the trap plants from the Jacmar plots. If pots from two time periods yielded adequate infections, the data from these periods were combined into one reading. Any data greater than 60% incidence (prior to transformation for multiple infections) were eliminated based on greenhouse trials indicating that greater than 60% incidence yielded inaccurate estimates of race frequency (see RESULTS, Greenhouse experiments).

Disease incidence, measured from infections on the trap

plants in Hyak plots, was considered an estimate of interplot interference in 1991. Trap plants from the pure stands of Hyak and Stephens plots were used to estimate interplot interference in 1992. The disease incidence averaged over all three races in these plots was compared to the average for all other treatments.

The data for disease incidence were corrected for multiple infections using Gregory's multiple infection transformation (Gregory, 1948; Waggoner & Rich, 1981). The data for each race were then converted to a proportion of the total amount of infection for the three races, and plotted over time. The model of Leonard (1969b) and similar regression models were used in an attempt to estimate relative fitnesses of the races. The frequency of each race for the last visit of each year was statistically analyzed using Fisher's protected L.S.D. means comparison procedure. We chose the 0.10 level of significance as appropriate to the sensitivity of our methods for sampling the pathogen population. The Shannon index (H_w) was applied to the data to measure the relative race diversity within the pathogen population. The equation $H_w = -\Sigma p_i \ln(p_i)$ calculates the Shannon index, with p_i being equal to the frequency of the i th race (Leonard, 1992).

Disease assessment of field plots. Whole-plot disease assessments were done on 27 and 29 May 1991, and 25 May and 2 June 1992, at Moro and Pendleton, respectively. The

majority of plants were heading at this time (stage 10.1-10.5 on the Feekes scale). Assessment was done by two observers estimating the percentage of total leaf area covered by stripe rust lesions. For the 1991 data, which included all component pure stands, percent disease reduction was calculated relative to the mean disease severity of the component pure stands for each mixture. Contrasts were used to test the statistical significance ($P \leq 0.05$) of these reductions.

Evaluation of cultivar proportions. At physiological maturity in 1991, an area containing approximately 100 tillers was hand-harvested from the center two rows of each mixture plot at a location about midway between the inoculation site and the end of the plot. Through a series of tests (separation by chaff color, reaction of seed to phenol, and reaction of seedlings to races of the pathogen), we determined the proportion of each cultivar in the mixtures at the end of the growing season (Finckh & Mundt, 1992b). The frequency of each cultivar was statistically analyzed using Fisher's protected L.S.D. means comparison procedure. Due to time and funding limitations, these procedures were not carried out in 1992.

CHAPTER III

RESULTS

Greenhouse experiments

Disease incidence on plants was proportional to the amount of inoculum used (Table 3). Estimated race frequency was independent of inoculation concentration except at the highest concentration of 0.04 g spores/pot. Disease incidence was above 60% (prior to transformation for multiple infections) at this concentration. For the other concentrations, there was no significant difference ($P \leq 0.05$) in incidence of disease caused by the three races (Table 3).

The second experiment showed that, when the three races were inoculated at unequal proportions, those proportions were reflected in the disease incidence readings. The two races inoculated at the same proportion were not significantly different ($P \leq 0.05$) in all but one case, and the race inoculated at twice their proportion was significantly different from the other two races (Table 4). There was some random variation between the two runs of the experiment with regard to measured frequencies, but no obvious bias in overestimating or underestimating the frequency of any race. Thus, the differences in genetic background of our differential cultivars did not seem to bias our estimates of race frequencies in the field. One of

the cultivars used in this experiment, Hyak, gave inconsistent results in its reaction to race 27 and the data from this cultivar were not used.

Field experiments

Interplot interference. At Pendleton in 1991, interplot interference ranged from 0 to 30%. At Moro, the range was 0 to 5%. Although the level of interference was higher at Pendleton than at Moro, treatment effects were similar between locations (see below). Hyak was believed to be resistant to all three races, but race 27 caused a very low level of infection in Hyak plots in 1991 (Table 7). In 1992, a pure stand of the cultivar Stephens was added as another control, and the number of buffer plots was increased. No stripe rust lesions were found in any of the Stephens plots, and no infections were found on the trap plants from either the Stephens or the Hyak plots in 1992.

Frequency of races. There was a great degree of variability in the frequencies of the three races at the first sampling period (Figures 1-4). However, this variability sorted out into distinct treatment differences over time (Figs. 1-4). The model of Leonard (1969b) and similar regression models often yielded nonsensical estimates of race fitnesses. This result was probably due to the small number of sampling points over time and our inability to find a single model to appropriately fit the data from all treatments. Therefore, we used the race frequencies at the last sampling as a

measure of the influence of the host treatments on the pathogen populations (Table 5).

The components of the mixtures determined whether or not the complex race was the most frequent. In the mixture JRY in 1991, a complex race interacted with two simple races, and simple races were most frequent. The complex race interacted with two compatible simple races in the mixture JPY. In this case, the complex race, race 29, was the most frequent race in three of four cases, although this difference was only significant ($P \leq 0.10$) in one case. In the mixtures JRH and JPH, the substitution of the cultivar Hyak for Tyee prevented race 27 from infecting these mixtures. The complex races were then the most frequent ($P \leq 0.10$) in all cases, except at Pendleton 1992 for the mixture JPH (Table 5). The Shannon index (Table 6) supports these conclusions. The index values averaged over both years for each location show that diversity was lowest for J, JPH, and JRH, and highest for JPY, JRY, and HPRY. The calculated index varied considerably for the mixture JPR. The overall low values for the Shannon index were due to the fact that only three races were used in the study.

Two treatments allowed a comparison of the relative fitnesses of the races when no race could infect more than one component of the host population. In the pure stand of the cultivar Jacmar, races 29 and 41 were both simple races infecting the same cultivar. For all visits except

Pendleton 1992, all three races were significantly different, with race 29 being in the highest proportion, suggesting that race 29 had a higher rate of reproduction than race 41 on this cultivar. The HPRY mixture allowed a comparison of the three races when each could infect only one cultivar in the mixture. This treatment provided inconclusive data for race frequencies in 1991, the only year when adequate infection was obtained for analysis.

Year and/or location had three qualitative influences on race frequency. First, when both 29 and 41 were complex races infecting the same mixture (JPR), location had a greater effect on the proportions of the two races than did the genetic background of the pathogen. Race 41 was the most frequent at Pendleton in both years, and race 29 was the most frequent at Moro in both years, although these differences were only significant for Moro 1992 (Table 5). Second, race 27 was more predominant in 1991 than in 1992, especially at the beginning of the season (Figs. 1-4). Third, the differences in the frequencies of races 29 and 41 were larger in 1991 than in 1992 on the pure stand of the cultivar Jacmar. In 1991, race 29 was more than twice as frequent as race 41, with the differences between the races being significant for both locations. Although race 29 was also more frequent than the other two races in 1992, the magnitude of the difference was smaller, and the difference was not significant at Pendleton (Table 5).

Disease severity. In 1992, disease severity was lower at Pendleton, and higher at Moro, as compared to 1991 (Table 7). There was high variation in the level of disease reduction among mixtures. However, the rankings of the mixtures for disease reduction were nearly identical at the two locations. The mixtures in which the complex races dominated (JPH and JRH) and the mixture JPR, in which both races 29 and 41 are complex, had the lowest disease reduction. For the other three mixtures, in which the complex races either were not the most frequent, or as in the case of HPRY, no race was complex, disease reduction was higher. Contrasts between the mean disease severities of the pure stands and the mixtures for 1991 were significant ($P \leq .05$) for all of the mixtures except JPH.

Changes in cultivar proportions over the growing season. Significant shifts in the proportions of the cultivars at harvest were not detected for most mixtures in 1991. The mixtures HPRY, JRY, and JPY at Pendleton, and the mixture JRY at Moro showed significant differences in cultivar proportions (Table 8). However, in none of these cases were the frequency shifts of the cultivars related to the measured pathogen race frequencies.

CHAPTER IV

DISCUSSION

The complex races in the mixtures did not always dominate the pathogen population. Rather, composition of the host and the number of compatible pathogen races present had the greatest influence on the frequencies of the three races. A complex race was significantly most frequent when it and only one other race could infect the mixture. In most cases, when the resistant cultivar Hyak was replaced with Tyee, which is susceptible to race 27, the complex race did not differ significantly in frequency as compared with the other two races. For example, in the mixture JRY in 1991, the frequency of race 27 was high, and the complex race 41 had no advantage over race 29. In 1992, however, when the frequency of race 27 was lower, there was a statistically non-significant trend for race 41 to be more frequent. Thus, increasing the number of virulent races in a mixture reduced the dominance of the complex race. The mechanism by which this interaction occurred is unknown, but could be related to an induced resistance phenomenon. Race 27 is avirulent on the cultivars attacked by the other two races, and induced resistance has been demonstrated for stripe rust (Johnson, 1978).

Plant pathologists are steadily gaining data from the field to understand mechanisms that determine the level of

disease seen in cultivar mixtures. Studies of these mechanisms have included reduced host density, barrier effects of resistant plants, and induced resistance (Chin & Wolfe, 1984a); interactions between components of race-nonspecific resistance (Jeger et. al., 1981a & 1981b); shifts in frequency of differentially susceptible cultivars in mixtures (Finckh & Mundt, 1992a); and alterations in host susceptibility due to competitive interactions between cultivars (Finckh & Mundt, 1992b). The positive correlation between disease control and pathogen diversity found in our study suggests that the race structure of the pathogen population is another important mechanism to be considered.

In practice, it is impossible to predict which pathogen races will be present at the time when farmers would need to decide which cultivars to grow in a mixture. However, this is true whether those cultivars are being grown in pure stands or mixtures. Indeed, one of the benefits of mixtures seems to be reliable yield among seasons in part due to compensation by one cultivar for the poor performance by another (Finckh & Mundt, 1992a; Wolfe and Barrett, 1980).

Marshall (1989) noted that the future evolutionary development of the pathogen population can only be determined by long-term studies of crop mixtures grown over large areas. In this regard, it is significant that environment seemed to influence race frequencies in our studies. Random environmental variation influenced the

initial establishment of the races. In fact, the relative fitnesses of *P. striiformis* races in Oregon are commonly observed to change ranking between years and locations (Mundt, unpublished). Both location and year had an influence on the final race frequencies measured in some of the mixtures of this study. Selection in heterogeneous environments is considered by some population geneticists to be an important mechanism to explain the existence of genetic variation in nature (Falconer, 1960). Thus, although a complex race may dominate in one year or location, it could be at a selective disadvantage in another year or location. Finally, our study did not address the effect of the overseasoning environment on pathogen fitness, a factor crucial to the long-term frequency of a pathogen genotype.

The clonal nature of many plant pathogens makes assessment of fitness effects of a trait very difficult. For example, if the relative fitness of a complex race is less than that of a simple race, it is unclear if this reduced fitness is due to "unnecessary virulence" or to some other aspect of the pathogen's genetic background (Leonard, 1977; Vanderplank, 1975). In our study, we altered the composition of wheat cultivar mixtures such that the same pathogen race would be either complex or simple, depending on the mixture in question. Although the results from the mixture JRY in 1991 show no significant evidence for

selection of complexity, the results from the mixture JRH do show evidence of selection for the complex race. The genetic background of the pathogen races also influenced whether or not the complex race dominated. When race 29 was the complex race, as in the mixtures JPY and JPH, it was more frequent than race 41 in all but one case, although this difference in frequency was not always significant. Whether or not race 41 was the most frequent when it was the complex race was influenced more by the components of the mixture and the year of observation. Conclusions concerning selection for complex races depend on the system studied, in this case the pathogen races present and the cultivars used in the mixtures.

Our results suggest that factors such as environmental interactions, genetic background of the pathogen race, host composition, and interaction among pathogen races may play as important a role in determining race frequencies in mixtures as does stabilizing selection. Our results do not rule out a role for stabilizing selection in preventing complex races from dominating the pathogen population in mixtures. Clearly, however, an evaluation of pathogen race dynamics in mixtures needs to progress beyond the simplistic analysis of the stabilizing selection concept that has received primary emphasis in plant pathology for considerable time.

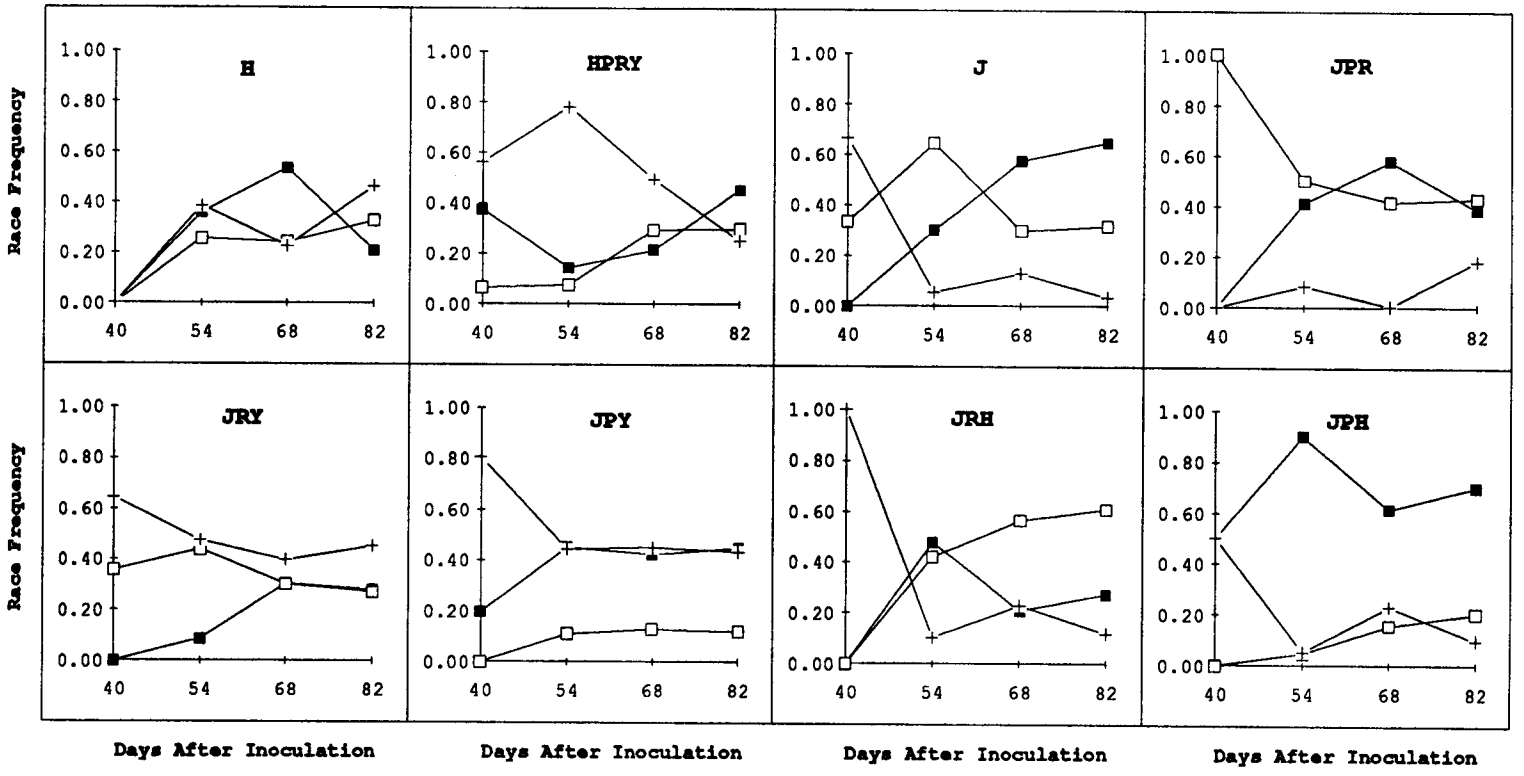

Figure 1. Proportional distribution of three races of *Puccinia striiformis* in wheat cultivar mixtures grown at Pendleton in 1991. H, J, P, R, and Y indicate the cultivars Hyak, Jacmar, Paha, Tres, and Tye respectively. The symbol ■ represents race 29, □ represents race 41, and + represents race 27.

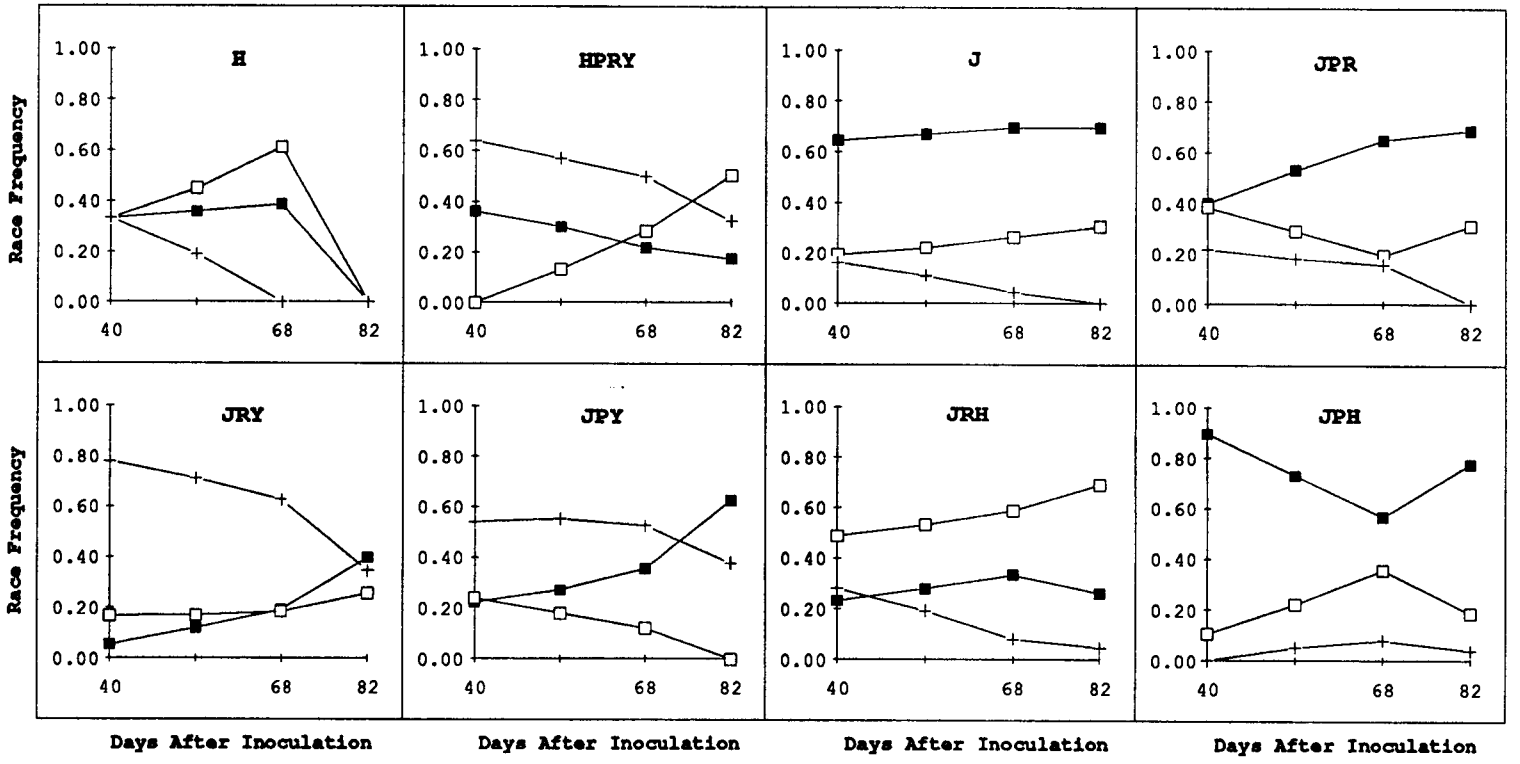

Figure 2. Proportional distribution of three races of *Puccinia striiformis* in wheat cultivar mixtures grown at Moro in 1991. H, J, P, R, and Y indicate the cultivars Hyak, Jacmar, Paha, Tres, and Tyee, respectively. The symbol ■ represents race 29, □ represents race 41, and + represents race 27.

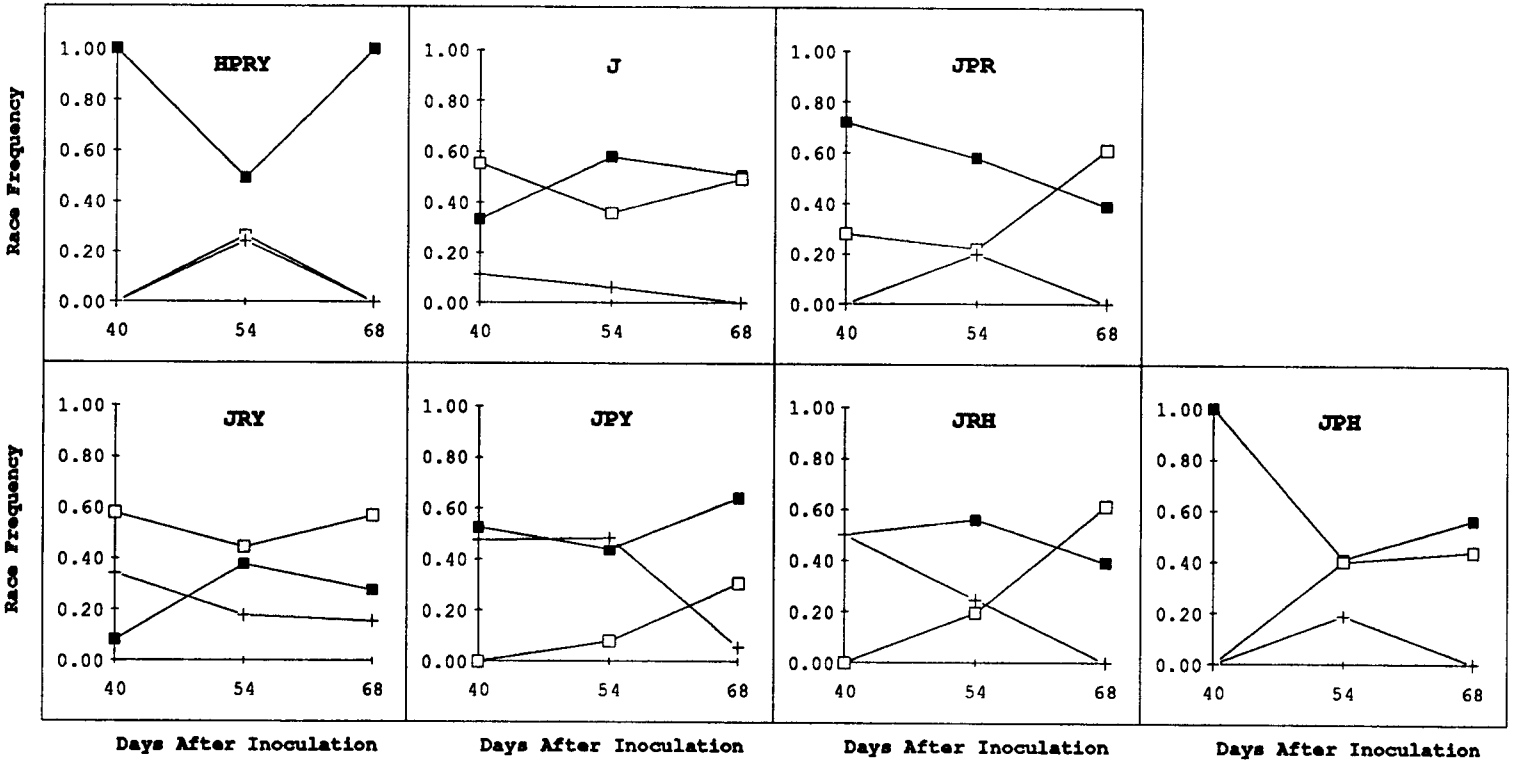

Figure 3. Proportional distribution of three races of *Puccinia striiformis* in wheat cultivar mixtures grown at Pendleton in 1992. H, J, P, R, and Y indicate the cultivars Hyak, Jacmar, Paha Tres, and Tyee, respectively. The symbol ■ represents race 29, □ represents race 41, and + represents race 27.

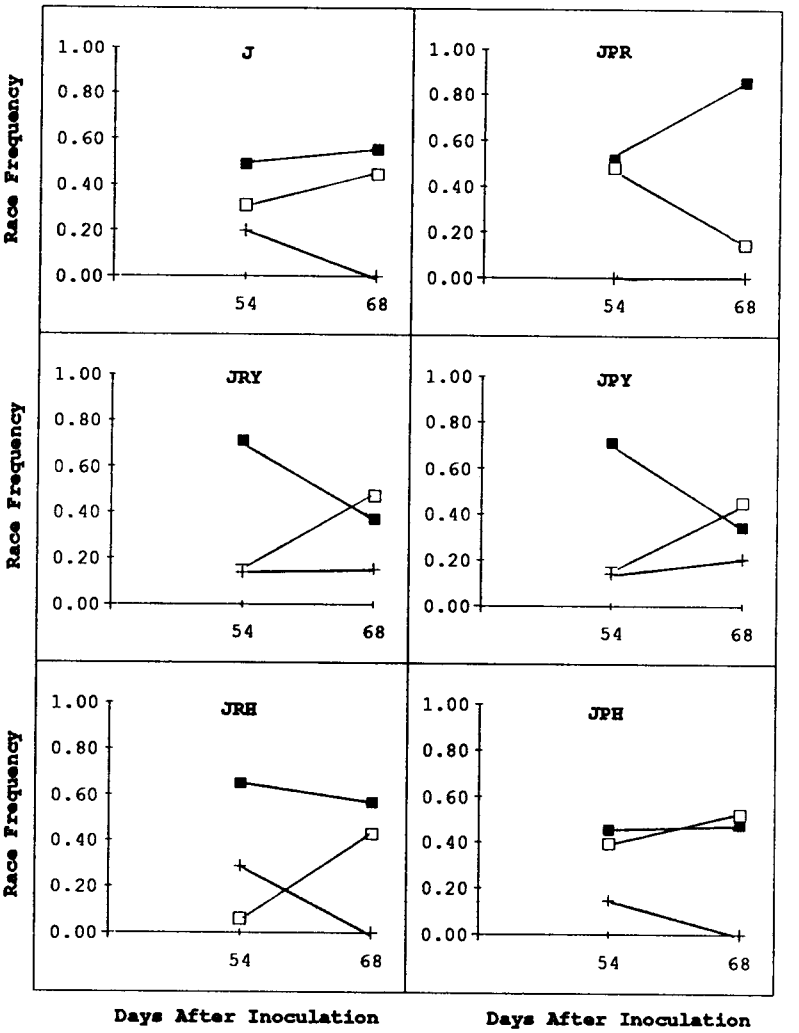

Figure 4. Proportional distribution of three races of *Puccinia striiformis* in wheat cultivar mixtures grown at Moro in 1992. H, J, P, R, and Y indicate the cultivars Hyak, Jacmar, Paha, Tres, and Tyee, respectively. The symbol ■ represents race 29, □ represents race 41, and + represents race 27.

Table 1. Compatibility between five cultivars of winter wheat and three races of *Puccinia striiformis* [a]

Cultivar	USDA accession number	Races [b]		
		CDL27	CDL29	CDL41
Hyak(H)	PI 511674	-	-	-
Jacmar(J)	NSL 95258	-	+	+
Paha(P)	PI 144485	-	+	-
Tres(R)	PI 17917	-	-	+
Tyee(Y)	PI 17773	+	-	-
Stephens(S)	PI 17596	-	-	-

[a] "-" indicates incompatibility and "+" indicates compatibility between cultivar and race

[b] CDL race designations are those of the USDA Cereal Disease Laboratory, Pullman, WA, USA.

Table 2. Wheat cultivars, cultivar mixtures, and *P. striiformis* races used in the field experiments

Cultivars and Mixutures [a]	Virulent Races
S [b]	none
H	none
J	29,41
HPRY	27,29,41
JPR	29*,41*
JRY	27,29,41*
JPY	27,29*,41
JRH	29,41*
JPH	29*,41

[a] S, H, J, P, R, and Y indicate the cultivars Stephens, Hyak, Jacmar, Paha, Tres, and Tyee, respectively

[b] 1992 season only.

* Indicates a complex race, i.e., a race capable of infecting two cultivars in that mixture.

Table 3. Effect of inoculum concentration on disease incidence caused by three *Puccinia stiiformis* races (Races 29, 41, and 27) when inoculated in equal proportions on differentially susceptible wheat cultivars (Paha, Tres, Tyee, and Orin) in the greenhouse

Inoculum conc. (g/pot)	% Disease incidence [x]			Race frequency [y]		
	Race 29	Race 41	Race 27	Race 29	Race 41	Race 27
0.0004	20.59	17.68	17.95	0.326 a[z]	0.285 a	0.389 a
0.001	15.71	20.21	9.45	0.331 a	0.317 a	0.352 a
0.004	50.67	51.94	47.03	0.342 a	0.351 a	0.307 a
0.02	50.73	43.51	50.39	0.303 a	0.383 a	0.314 a
0.04	52.90	80.75	53.12	0.239 a	0.511 b	0.250 a

[x] Percent disease incidence was based on the proportion of second and third leaves that had sporulating lesions. Since the average percent disease incidence of all three of the races at the 0.04 concentration was above 60%, this value was used as a maximum value for the field data.

[y] Each race could infect only one of the differential cultivars. Race frequency was based on disease incidence corrected by Gregory's multiple infection transformation (Gregory, 1948)

[z] Means within a row followed by the same letter do not differ significantly (P =0.05) according to Fisher's Protected L.S.D. means comparison procedures.

Table 4. Incidence [y] of stripe rust on differential wheat cultivars (Paha, Tres, Tyee, and Hyak) in the greenhouse inoculated with a mixture of three races of *Puccinia striiformis*

	Ratio of races 29:41:27		
Trial/Race	2:1:1	1:2:1	1:1:2
Trial 1			
Race 29	0.66 b [z]	0.26 a	0.20 a
Race 41	0.18 a	0.50 b	0.20 a
Race 27	0.16 a	0.24 a	0.60 b
Trial 2			
Race 29	0.59 b	0.12 a	0.28 a
Race 41	0.25 a	0.56 c	0.29 a
Race 27	0.16 a	0.32 b	0.43 b

[y] Each race could infect only one of the differential cultivars. Pots were inoculated with a total of 0.02 g inoculum/pot. Race frequency was based on disease incidence corrected by Gregory's multiple infection transformation.

[z] Means within a column followed by the same letter do not differ significantly ($P \leq 0.05$) according to Fisher's Protected L.S.D. means comparison procedures.

Table 5. Proportions of three *P. striiformis* races sampled near the beginning of crop maturation (stages 10.1-10.5 on the Feekes scale) in different wheat cultivars and mixtures, in two years and locations (Moro and Pendleton, OR).

		Location and Year			
Treatment[x]	Race	Pend. 1991	Moro 1991	Pend. 1992	Moro 1992
J	27	0.035 a [y]	0.000 a	0.000 a	0.000 a
	41	0.317 b	0.305 b	0.490 b	0.445 b
	29	0.650 c	0.695 c	0.510 b	0.555 c
HPRY	27	0.250 a	0.322 a	------ [z]	------
	41	0.300 ab	0.502 a	------	------
	29	0.450 b	0.173 a	------	------
JPR	27	0.183 a	0.000 a	0.000 a	0.000 a
	41*	0.430 a	0.312 ab	0.607 b	0.144 a
	29*	0.387 a	0.688 b	0.393 b	0.857 b
JRY	27	0.450 a	0.345 a	0.155 a	0.153 a
	41*	0.270 a	0.255 a	0.567 b	0.470 b
	29	0.278 a	0.400 a	0.278 ab	0.375 b
JPY	27	0.435 b	0.377 ab	0.058 a	0.205 a
	41	0.117 a	0.000 a	0.305 a	0.450 a
	29*	0.450 b	0.623 b	0.637 b	0.345 a
JRH	27	0.120 a	0.048 a	0.000 a	------
	41*	0.610 c	0.690 b	0.610 c	------
	29	0.275 b	0.263 a	0.390 b	------
JPH	27	0.100 a	0.037 a	0.000 a	------
	41	0.203 a	0.183 a	0.438 b	------
	29*	0.700 b	0.778 b	0.562 b	------

[x] J, H, P, R, and Y indicate the cultivars Jacmar, Hyak, Paha, Tres, and Tyee, respectively.

[y] Values for each treatment (within a column) that differ significantly ($P \leq 0.1$) according to Fisher's Protected L.S.D means comparison procedures are denoted by different letters within each location/year combination.

[z] Dotted lines indicate missing data.

* Indicates a complex race, i.e., a race capable of infecting two cultivars in the mixture.

Table 6. Shannon index [a] values for diversity of *Puccinia striiformis* populations in a pure stand and six different mixtures of wheat cultivars in two locations and years

	Location and Year					
	Pendleton			Moro		
Trtmnt[b]	1991	1992	Mean	1991	1992	Mean
J	0.762	0.693	0.728	0.615	0.687	0.651
HPRY	1.067	xxx[c]	1.067	1.014	xxx	1.014
JPR	1.041	0.670	0.856	0.621	0.411	0.516
JRY	1.069	0.967	1.018	1.082	1.010	1.046
JPY	0.972	0.815	0.894	0.663	1.051	0.857
JRH	0.911	0.669	0.790	0.753	xxx	0.753
JPH	0.804	0.685	0.745	0.628	xxx	0.628

a Larger values of the Shannon index indicate greater diversity.

b Treatments abbreviated as J, H, P, R, and Y indicate the cultivars Jacmar, Hyak, Paha, Tres, and Tyee, respectively.

c Indicates a missing value.

Table 7. Severity of yellow rust at heading (stages 10.1-10.5 on the Feekes scale) for wheat cultivars and mixtures, and reduction of yellow rust in mixtures relative to their component pure stands in field plots in two locations and years

	%Disease severity[a]		%Disease reduction[b]	
	Location		Location	
Year/ Treatment[c]	Pendleton	Moro	Pendleton	Moro
1991				
H	1.03	0.00	-----	-----
J	44.38	8.40	-----	-----
P	38.13	8.03	-----	-----
R	39.38	7.60	-----	-----
Y	51.13	10.13	-----	-----
HPRY	11.13	3.55	65.30	44.22
JPR	27.13	5.55	31.50	32.11
JRY	18.50	5.03	58.20	43.78
JPY	26.63	5.50	41.18	37.62
JRH	17.63	3.85	31.56	25.92
JPH	22.25	4.50	10.48	20.07
1992	Pend.	Moro		
H	0.00	2.33		
J	32.88	27.38		
HPRY	1.63	4.75		
JPR	2.63	6.50		
JRY	7.75	10.00		
JPY	6.88	11.25		
JRH	1.00	5.63		
JPH	3.40	4.88		
S	0.00	0.00		

[a] Disease severity was estimated as the percentage of total leaf area covered by yellow rust lesions, on a whole plot basis.

[b] Disease reduction was calculated relative to the mean of the component cultivars grown in pure stands.

[c] J, H, P, R, and Y indicate the cultivars Jacmar, Hyak, Paha, Tres, and Tyee, respectively.

Table 8. Wheat cultivar proportions in mixtures at the end of the 1991 growing season as determined by seperation by head color, reaction to phenol, and reaction to races of *P. striiformis*

Mixture/ Components	Location	
	Pendleton	Moro
HPRY		
Hyak	15.3 a [z]	------
Paha	28.9 ab	------
Tres	24.9 ab	------
Tyee	30.9 b	------
JPR		
Jacmar	28.9 a	34.6 a
Paha	37.2 a	34.2 a
Tres	33.9 a	31.2 a
JRY		
Jacmar	26.5 a	22.2 a
Tres	26.1 a	46.9 b
Tyee	47.4 b	30.9 ab
JPY		
Jacmar	23.8 a	26.9 a
Paha	46.4 b	41.3 a
Tyee	29.8 a	31.8 a
JRH		
Jacmar	22.4 a	38.2 a
Tres	45.0 a	34.3 a
Hyak	32.6 a	27.5 a
JPH		
Jacmar	26.2 a	39.4 a
Paha	39.8 a	37.3 a
Hyak	34.0 a	23.3 a

[z] Values within a column for each mixture/location combination that differ significantly ($P = 0.05$) according to Fisher's Protected L.S.D means comparison procedures are denoted by different letters

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